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A Study of Ylide Extractions of Mercury in Fish and Water Using Cold Vapor Flameless Atomic Absorption Techniques

Howard Vail

A STUDY OF YLIDE EXTRACTIONS OF MERCURY IN FISH AND WATER USING COLD VAPOR FLAMELESS ATOMIC ABSORPTION TECHNIQUES

A Thesis

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Master of Science

by

Howard P. Vail

May 1980

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A STUDY OF YLIDE EXTRACTIONS OF MERCURY
IN FISH AND WATER USING COLD VAPOR
FLAMELESS ATOMIC ABSORPTION TECHNIQUES

Recommended May 5, 1980
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A STUDY OF YLIDE EXTRACTIONS OF MERCURY
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FLAMELESS ATOMIC ABSORPTION TECHNIQUES

Howard P. Vail May 1980 60 pages
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Cold vapor flameless atomic absorption spectrophotometry is an analytical method used in the determination of mercury. Its sensitivity is less than one part per billion using a 100 milliliter sample, and there are virtually no interferences from other metal ions. The method is best carried out making use of a permanganate-persulfate oxidation step with heat.

The ylide, triphenylphosphoniumcyclopentadienylide ($CpPPh_3$), which extracts mercury almost quantitatively from water, was used in attempts to remove mercury from exposed goldfish. Though not toxic to the fish, $CpPPh_3$ did not significantly lower mercury levels. Evaluation of $CpPPh_3$ as a possible phase transfer catalyst failed to identify a true catalytic species.

I. INTRODUCTION

A. Mercury and its Effects on Fish

Mercury was known to ancient civilizations and is a modern problem. Its toxic properties were known by Hippocrates around 400 BC. Mercury compounds are found in trace quantities throughout the environment and pose no threat to health. However, concentration of mercury via industrial processes and its discharge into the environment poses a serious problem. It is known that fish, for example, have a tendency to concentrate mercury.

In areas where industrial effluents have distributed large amounts of mercury, fish have potentially dangerous mercury concentrations, as evidenced by the Minamata, Japan, disaster in the early 1950's. The cause of this epidemic was traced to consumption of fish which contained high concentrations of alkylmercury compounds. Victims were stricken with neurological illnesses characterized by weakening of the muscles, partial blindness, impairment of other cerebral functions, paralysis and even death.

A property unique to mercury is its ability to form stable compounds with organic radicals. The number of such compounds is so large that mercury is said to have an organic chemistry of its own. Broadly, these may be divided into three groups: alkyl, aryl, and alkoxyaryl.

The transformation of mercury compounds varies a great deal in aquatic environments; it may, for example, be methylated or precipitated. Mercury in sediment is often in the form of the highly insoluble sulfide and it remains there under anaerobic conditions. However, under aerobic conditions oxidation to the more soluble sulfate may occur and the mercury may then enter into the methylation process, illustrated in Figure 1. Inorganic, aryl and alkoxyaryl mercury compounds can all be transformed to methylmercury,[2,3] although Matsumura et al.[4] found that both aquatic and soil microorganisms convert phenylmercuric acetate to dipnenylmercury, and no methylmercurials were found.

Fish exposed to mercury in water exhibit rapid and efficient mercury uptake. Hannerz[5] examined the concentration of mercury by fish in a pond situation. Cod exposed to mercury for two days showed concentration factors of 388 for methoxyethylmercuric hydroxide and 606 for methylmercuric hydroxide. After one month the concentration factors for inorganic and alkoxyalkyl mercury compounds were similar and of the lowest amount, while the highest values were for alkylmercury compounds. McKone et al.[6] showed that mercuric ion is rapidly absorbed by goldfish. Fish exposed to 50 liters of a solution 0.25 ppm in mercuric ion concentrated mercury by absorption to the extent of 40 to 50 ppm in 100 hours (see figure 2). The mercuric ion is rapidly absorbed into the mucus externally secreted by goldfish and its presence seems to stimulate this secretion. It also is evident that mercury concentrates in the fish liver. Great Blue Heron with carcass levels of 21.2-23.0 ppm mercury had

Figure 1. Natural Methylation of Mercury in Waterways[1]

AIR

Evaporation and precipitation cycle naturally to the atmosphere from land and water with industrial pollution added.

LAND

Weathering, land runoff and industrial waste are discharged to the waterway.

WATER

Mercury compounds degrade into one of three forms: Elemental, Mercuric ion or Mercurous ion which converts into one of the other two depending on the pH of the waterway.

SEDIMENT

The mercury adsorbs on particles and sirks to the sediments where it is stored or methylated

NATURAL METHYLATION

TRAGEDY

Human beings, fish eating birds and other mammals eat the fish, concentrate the methylmercury further and may be poisoned quickly or very gradually.

CONCENTRATION

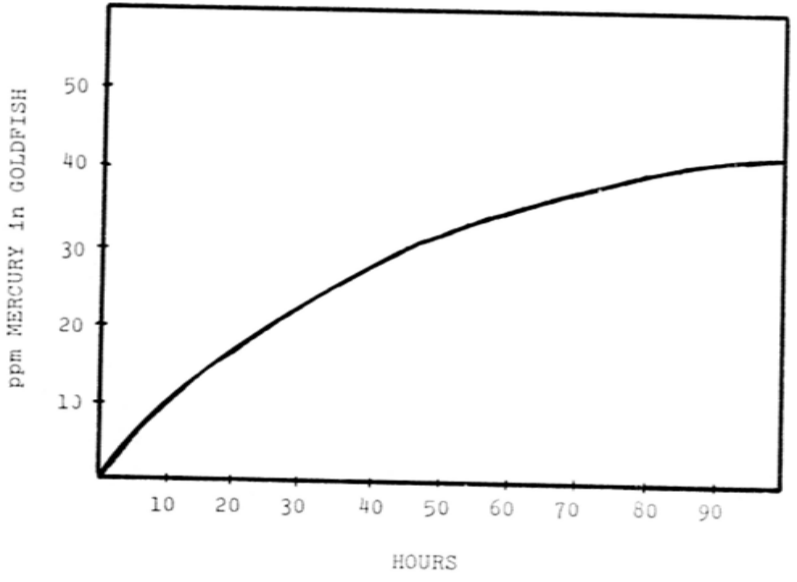
Very small quantities of mono- and dimethylmercury released to the water by the microbes are absorbed and ingested by the fish to concentrate to steadily higher levels.

METHYLATION

Microbes on the surface of the sediments methylate elemental mercury in anaerobic conditions and mercuric ion in aerobic waters.

Living microbes methylate mercury biologically while components of dead microbes methylate it chemically.

Figure 2. Concentration of Mercury Absorbed by Goldfish from a Solution Containing 0.25 ppm of Mercury as $HgCl_2$ as a Function of Time[6]


ppm MERCURY in GOLDFISH
50
40
30
20
10
10
20
30
40
50
60
70
80
90
HOURS

136-175 ppm mercury in the liver, while they had fish in their stomachs containing only 1.8-3.6 ppm mercury.[7] Hannerz also found that after 4 weeks the percentage decrease of mercury in pike previously exposed was 30% for blood, 17% for muscle, 13% for liver and 0% for bone.

B. Methods of Removing Mercury from Water

By the time elevated mercury levels were discovered in fish, many waterways had accumulated appreciable amounts of mercury in both the water and the sediment. These high mercury levels prompted the need to find methods for permanent recovery of the mercury and to ban its further discharge into the waterways.

If the mercury in the sediments is buried, then it is not subject to biological methylation. Natural sedimentation is the preferred method for burying mercury but it normally takes about 5 years to accumulate a 1.5 inch layer of sediment.[8] Sand and gravel could also be spread over the sediments. Inert clays and freshly ground silicate also bind with mercury to form a stable covering and water currents would be less apt to disrupt it.[9]

There are several natural chelating polymers that have been proposed for use in removal of mercury from waters. Among these are polyamino acids,[10] sulfide-treated polyurethane foams[11] and chitosan.[12] However, they are all relatively expensive and are effective in removing only inorganic mercury. Polymer films might also be used to cover the sediments, but their installation, cost and tearing by strong currents severely limit their applicability.

Thiols, probably the best chelating agents for mercury, are oily liquids that impart foul odors and tastes to fish. They also float on water and would have to be sunk in order to react with mercury in the sediment. They are fairly costly but only small amounts are needed.[13] A current use of thiols involves incorporating vicinal thiols grafted onto a cellulose matrix; the absorbent may then chelate mercury.[14]

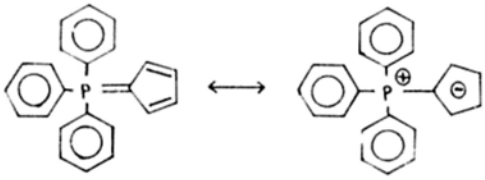
A number of other substances have been tested and found to bind to mercury. Among these are nut wastes[15] and naval stores products such as tall oil soap.[16] Cotton fibers that are chemically modified with nitrogen compounds also extract mercury from solution,[17] as do keratin derivatives of wool.[18] Methods useful in removing mercury from drinking water include use of anion exchange resins,[19] tire rubber,[20] zinc filters[21] and activated carbon.[22,23]

Most of the above processes are expensive and some remove only part of the mercury. They may also cause additional waste removal problems. Therefore, dredging would seem to be the most obvious way to remove mercury-laden sediments from water bodies, but it is also one of the most controversial.[13,24] When dredging is feasible, new techniques need to be devised to stir up as few sediments as possible thus preventing mercury from being released back into the water. Possible alternatives to dredging include dikes and open-lake disposal.

C. Triphenylphosphoniumcyclopentadienylide as a Chelating Agent for Mercury

The ylide triphenylphosphoniumcyclopentadienylide ($CpPPh_3$)

was first synthesized by Ramirez and Levy[25] in 1956. The distribution of the negative charge over the cyclopentadienide



ring confers a high degree of stability on this structure. It exists as yellow crystals (m.p. 229-231°C) and is quite insoluble in water, but soluble in dilute mineral acids.

Cationic mercury halide complexes of $CpPPh_3$ have been synthesized and characterized.[26] The 1:1 adduct $CpPPh_3HgI_2$ (m.p. 191-192°C dec.) is shown in Figure 3. The iodide, bromide and chloride complexes all show long-term stability in the solid form. The coordination about the mercury is a distorted tetrahedron. In solution, a fluxional σ-bond between the mercury and the cyclopentadienide ring is favored.

Recent studies have shown that $CpPPh_3$ extracts mercury from aqueous solution selectively.[27] In solutions containing Fe, Cu, Zn, Mn and Pb, less than 3% of these metals were extracted in all cases (see Table 1). Mercuric ion was removed at greater than 98% efficiency over a wide pH range. $CpPPh_3$ also removed Cd but only to the extent of 7%. Thus the ylide shows promise in removing mercury without also coordinating ions which are physiologically important.[28]

D. Atomic Absorption Spectrophotometry and the Cold Vapor Flameless Techniques

In conventional flame atomic absorption spectrophotometry, a fine spray of the sample solution is introduced into a

Figure 3. ORTEP Diagram of $[(C_6H_5)_3PC_5H_4HgI_2]_2$ [26]

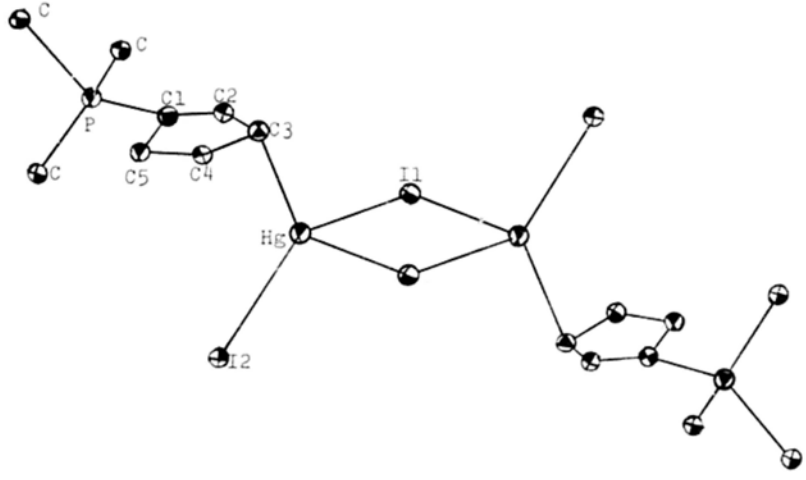

C
C
P
C1
C2
C3
C
C5
C4
I1
Hg
I2

TABLE 1. Selectivity of $CpPPh_3$ towards mercury.

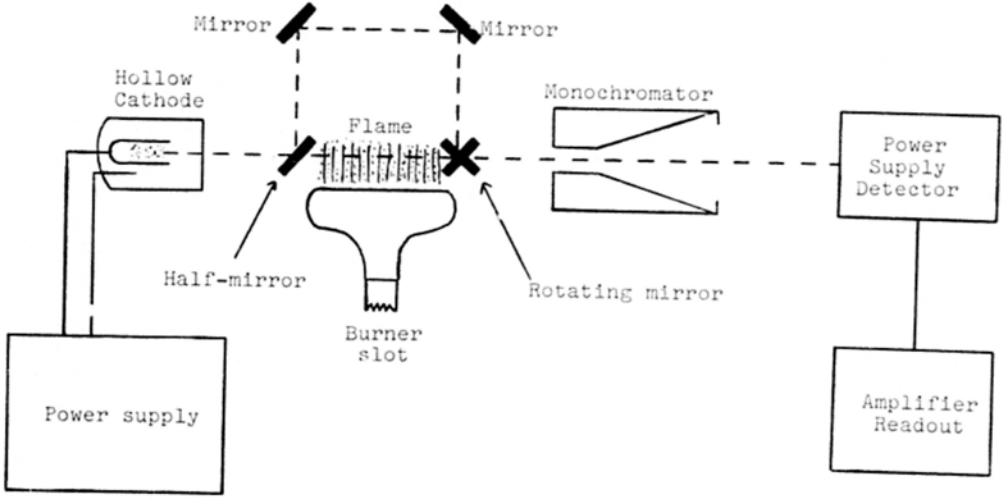
Metal	original solution [M] (ppm)	after extraction [M] (ppm)	%M removed
*Hg^{+2}	4.08	0.04	99.0
Hg^{+2}	497	<10	>98.0
Fe^{+3}	43.9	42.4	3.4
Fe^{+2}	46.9	46.6	0.6
Cu^{+2}	40.0	38.8	3.0
Zn^{+2}	4.01	3.97	1.0
Mn^{+1}	10.05	10.00	0.5
Pb^{+2}	48.6	48.3	0.6
Cd^{+2}	9.90	9.18	7.3
Ca^{+2}	48.7	48.2	1.0

*flameless method

flame where it is desolvated, vaporized and atomized. Radiation from an external light source, emitting the spectral line(s) that corresponds to the energy required for an electronic transition from the ground state to an excited state, is passed through the flame. The flame gases are treated as a medium containing free, unexcited atoms capable of absorbing radiation from an external source when the radiation corresponds exactly to the energy required for a transition of the test element from the ground electronic state to an upper excited level. Unabsorbed radiation then passes through a monochromator that isolates the exciting spectral line of the light source and into a detector. The absorption of radiation from the light source depends on the population of the ground state, which is proportional to the solution concentration sprayed into the flame. Absorption is measured by the difference in the transmitted signal in the presence and absence of the test element. A schematic diagram of the measuring technique is shown in Figure 4.

The cold vapor flameless atomic absorption method for mercury is a physical one based on the absorption of radiation at 253.7 nm by mercury vapor. The mercury is chemically reduced to the elemental state and aerated from solution in a closed system. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer. The absorbance is measured just as in conventional flame methods. The chief advantage of the flameless method lies in its sensitivity. Using a 100 ml sample, a detection limit of 0.2 µg Hg/l can be achieved, which is more than 100

Figure 4. Method of Analysis Used in Conventional Flame Atomic Absorption


Mirror
Mirror
Hollow
Cathode
Flame
Monochromator
Power
Supply
Detector
Half-mirror
Rotating mirror
Burner
slot
Power supply
Amplifier
Readout

times better than that of the dithizone method.[29] Most other metal ions do not interfere.[30]

The most common procedure for flameless analysis is that described by Hatch and Ott.[31] The sample is oxidized with $KMnO_4$ in the presence of nitric and sulfuric acids. $NH_2OH \cdot HCl$ and $SnCl_2$ are added to generate the mercury vapor which passes through an $MgClO_4$ trap and into the absorption cell. Uthe[32] describes an apparatus which improves reproducibility and may be used for partial sample automation.[33] A simple and precise method for transfer of mercury vapor is described by Stainton.[34] Another variation of the Hatch and Ott procedure has been adapted for the analysis of foods using HNO_3 decomposition,[35] and for urine, blood, water and air with HNO_3/H_2SO_4 digestion.[36,37] Malaiyandi and Barrette[38] decomposed biological materials with $H_2SO_4/HNO_3/V_2O_5$. Omang[39] has a method for water and industrial effluents using $H_2SO_4/KMnO_4$ digestion, and Goulden and Afghan[40] one for water with destruction of organic compounds by ultraviolet photo-oxidation. Chau and Saitoh,[41] in water analysis, acidify and then concentrate the mercury by means of a dithizone extraction followed by a back extract into a small volume. Lee and Laufmann[42] show that cellulose materials may be digested with aqua regia. The Dow Chemical Company has published details of methods for water, brines, caustic, fish, sludges, mud, hydrogen and air.[43]

In biological samples it is often important to be able to distinguish between inorganic mercury and methylmercury content, particularly in fish. Methods for the determination of total mercury in fish and other foods are numerous.[44-52]

These are based on the rapid conversion of organomercurials first into inorganic, divalent mercury and then into mercury vapor, the latter brought about by use of a combined $SnCl_2$ - $CdCl_2$ reagent. It was found that if $SnCl_2$ alone were added instead of the $SnCl_2$-$CdCl_2$ reagent, only the release of inorganic mercury influenced the absorbance reading, thus permitting the selective determination of inorganic mercury in the presence of methylmercury.[53,54] It was possible first to release inorganic mercury then, after re-acidification of the reaction mixture, methylmercury by adding the $SnCl_2$-$CdCl_2$ reagent and NaOH. When total mercury and inorganic mercury were determined separately, the difference between the two results gave the methylmercury content of the sample. When the results for total mercury for this method and a total mercury method were statistically compared using a paired t-test, the difference between the results obtained by the two methods was found to be insignificant at the 95% confidence level. The results of a study involving black marlin samples are shown in Table 2.

E. Statement of Purpose

It was the purpose of this work to test the effectiveness of the ylide triphenylphosphoniumcyclopentadienylide ($CpPPh_3$) in removing mercury from exposed goldfish. Cold vapor flameless atomic absorption was our method of choice for mercury analysis due to its wide applicability and high sensitivity; thus, it was necessary to develop a specific procedure for analysis of samples that best suited our needs.

Table 2. Comparison of the Selective Reduction and Permanganate Methods for Mercury (ppm) in Some Marlin Samples

Sample No.	Selective Reduction			Permanganate, total Hg	Methyl Hg from selective reduction, %
	Inorganic Hg	Methyl Hg	Total Hg		
1	0.14	0.18	0.32	0.39	56.2
2	0.02	0.38	0.40	0.47	95.0
3	0.02	0.09	0.11	0.11	83.3
4	0.02	0.45	0.47	0.43	96.6
5	0.12	0.20	0.32	0.42	62.5
6	0.14	0.21	0.35	0.30	60.0
7	0.10	0.22	0.32	0.43	68.7
8	0.27	0.36	0.63	0.98	57.1
9	0.21	0.29	0.50	0.86	58.4
10	0.28	0.32	0.60	0.79	53.3
11	0.14	0.24	0.38	0.33	63.1
12	0.19	0.27	0.46	0.45	58.7
13	0.09	0.11	0.20	0.22	55.0
14	0.11	0.20	0.31	0.30	64.5
15	0.21	0.41	0.62	0.60	66.1
16	0.20	0.32	0.52	0.53	61.5
17	0.27	0.50	0.77	0.85	64.9
18	0.05	0.18	0.23	0.21	78.3
19	0.12	0.18	0.30	0.33	60.0
20	0.23	0.47	0.70	0.57	67.1
21	0.08	0.33	0.41	0.43	80.5
22	0.02	0.51	0.53	0.49	96.2
23	0.07	0.12	0.19	0.20	61.6
24	0.11	0.21	0.31	0.35	66.4
25	0.21	0.27	0.48	0.40	56.7

Since $CpPPh_3$ is capable of removing mercury selectively and quantitatively from water, it was our hope that the exposed goldfish would ingest the $CpPPh_3$, which might chelate the mercury in their bodies and then be expelled. Goldfish were chosen for this study because they are easy to obtain in large numbers. Furthermore, the mercury uptake rate of goldfish had previously been studied. It was also of interest to evaluate $CpPPh_3$ as a possible phase transfer catalyst.

II. EXPERIMENTAL

A. Reagents and Glassware

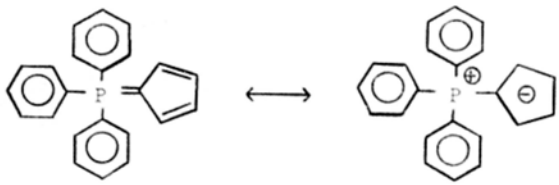
The following reagents were analytical grade: $HgCl_2$, PhHgCl, $KMnO_4$, $K_2S_2O_8$, $NH_2OH \cdot HCl$ and $SnCl_2$. Concentrated nitric and sulfuric acids of low mercury content, available from J. T. Baker Chemical Company, were used throughout the experiments. The water was deionized and distilled. Solvents used in the phase transfer catalysis studies were reagent grade.

The 1000 ppm Hg standard solution was prepared by dissolving 0.1354 g $HgCl_2$ in 75 ml deionized water, adding 10 ml concentrated HNO_3 and diluting to 100 ml. The solution was standardized using a Harleco atomic absorption mercury standard solution, also 1000 ppm in Hg. In addition, an organomercury standard was prepared by dissolving 0.1561 g PhHgCl in ethanol with heating, adding 100 ml concentrated HNO_3, and diluting to 1 ℓ with deionized water. The resulting solution was 100 ppm in Hg. All working solutions were prepared by dilution of the standard solutions, the HNO_3 content being maintained at 0.15% in all cases.

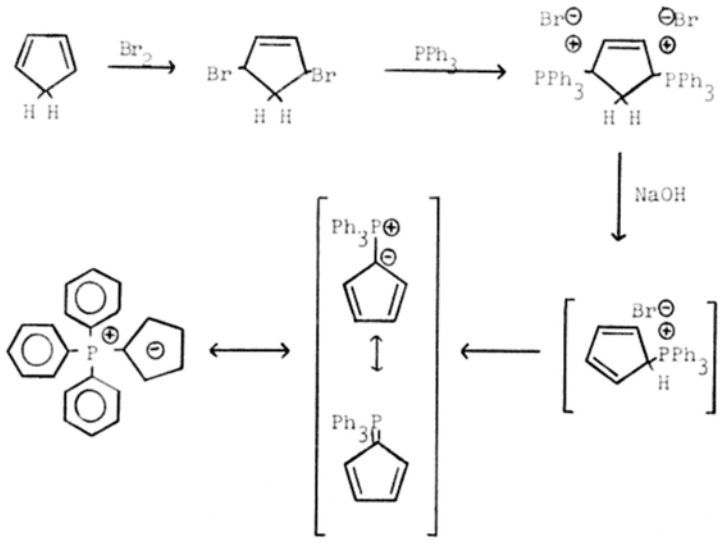
The triphenylphosphoniumcyclopentadienylide was prepared using the method of Ramirez and Levy (see Figure 5). The product, recrystallized from ethanol, was obtained as a tan solid (m.p. 228-229°C).

The BOD sample bottles used in the flameless analysis were cleaned by the following procedure:[55]

Figure 5. Triphenylphosphoniumcyclopentadienylide and its Synthesis[25]



Triphenylphosphoniumcyclopentadienylide



1. Wash thoroughly, using hot soapy water
2. Rinse twice with hot tap water
3. Wash twice with 50 ml concentrated HNO_3 (reagent grade)
4. Rinse twice with hot tap water
5. Rinse twice with deionized water, and invert to drain
6. Stopper using a piece of tissue between the stopper and the bottle.

It is to be stressed that all cleaning efforts which prevent mercury contamination are well worth the effort. Mercury is a common laboratory contaminant in air, dirt, dust, paper fibers, glassware, reagents and even on laboratory floors and furnishings.

B. Apparatus

Water bath shaker. All phase transfer catalysis experiments were performed in 250-ml glass bottles having a polyethylene cap and liner. The samples were agitated on a mechanical water bath shaker manufactured by Eberbach Corp., Ann Arbor, Michigan. The shaker consists of an internal thermostatted heater for controlling the temperature up to 100°C. The shaker speed can be adjusted up to about 260 shakes/minute with a 0.5 inch amplitude.

Aquaria and accessories. The two aquaria used in this study were manufactured by Jewel Aquarium Company, Chicago, Illinois, and each had a total capacity of about 78 gallons (296 ℓ). The aerators used (one per tank) were manufactured by Aquarium Tank Pump Inc., Prescott, Arizona.

Atomic absorption spectrophotometer and flameless accessories. A Perkin-Elmer model 303 Atomic Absorption Spectrophotometer was used as well as the mercury hollow cathode lamp which was purchased from the Perkin-Elmer Corp., Norwalk, Connecticut.

The Perkin-Elmer Mercury Analysis System is an accessory which permits the flameless atomic absorption measurement of mercury. The system is set up as shown in figure 6.

The operation of the system is quite simple. The circulating pump outputs air at the rate of 3 ℓ/minute from port A. The air flows through Tygon tubing to the aerator, which bubbles air through the reduced sample in the BOD bottle. The mercury vapor generated is passed through the lower aerator vent (Figure 7) to port B on the pump. The vapor immediately exits the pump at port D and passes through the dessicant tube to remove any water vapor. The mercury vapor is then swept into the absorption cell, a 155mm x 18mm cylindrical plastic tube equipped with replaceable plastic windows on the screw-cap ends. The cell is mounted on a holder which fits into the burner slot on the spectrophotometer, thus positioning the cell in the light path. The vapor passes through the cell, re-enters the pump at port C and is recirculated through port A. The mercury vapor becomes evenly distributed throughout the closed system. The maximum reading, which is usually achieved about 30 seconds after the aerator is connected, is recorded. To flush the mercury from the system, the tubing is removed from port C and the clamp is moved from point E to point F (Figure 6).

Figure 6. The Flameless Atomic Absorption Mercury Analysis System

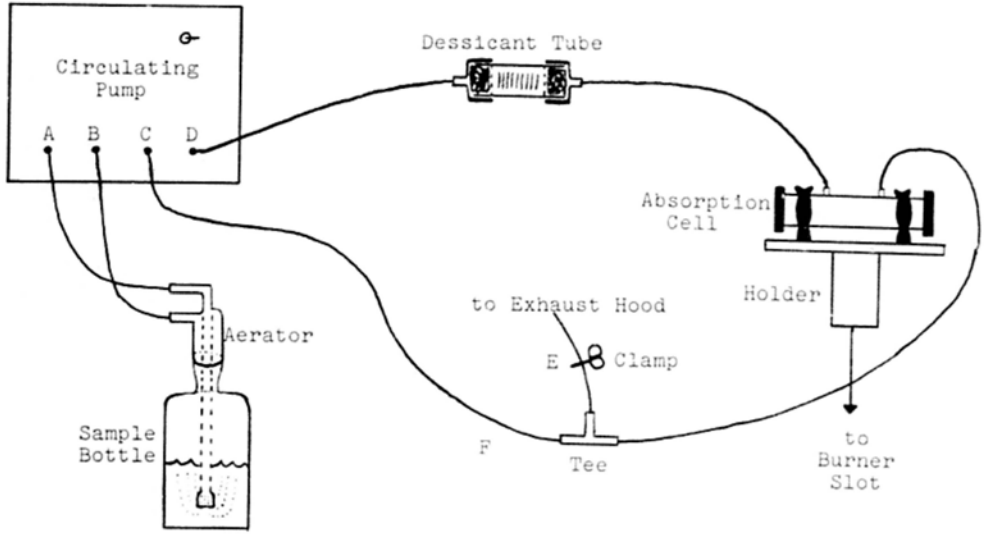
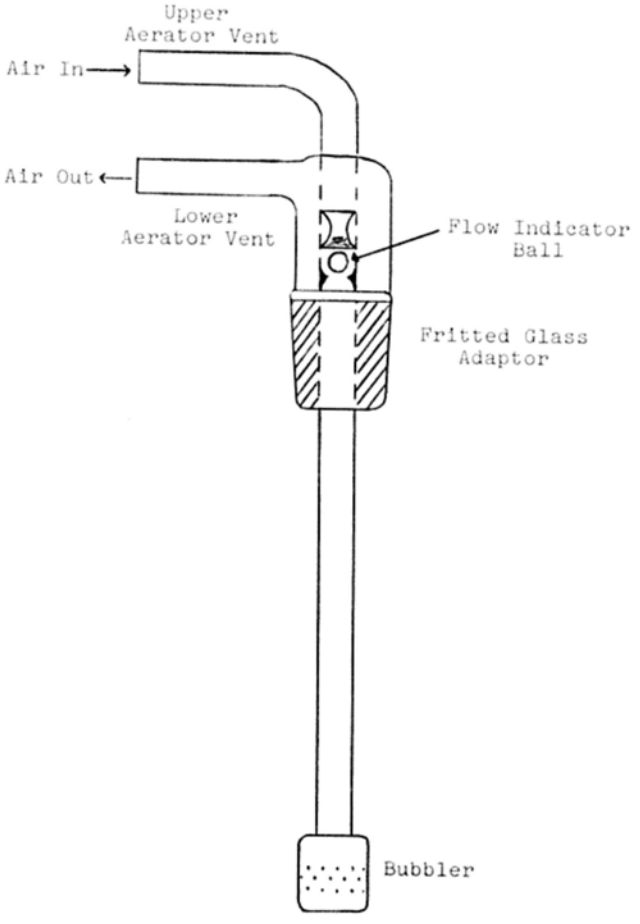

Circulating
Pump
A
B
C
D
Dessicant Tube
Absorption
Cell
Holder
to Exhaust Hood
E
Clamp
Aerator
Sample
Bottle
F
Tee
to
Burner
Slot

Figure 7. Full-Scale Drawing of the Aerator Tube


Upper
Aerator Vent
Air In
Air Out
Lower
Aerator Vent
Flow Indicator
Ball
Fritted Glass
Adaptor
Bubbler

The dessicant tube plays a very important role. That is, water vapor must be removed, for if it reaches the cell the incident light from the hollow cathode lamp will be scattered. This results in erroneously high readings. The tube is shown in Figure 8. Anhydrous $CaCl_2$, some of which is blue (turning to pink upon saturation with water), proved to be a satisfactory dessicant. It is also possible to use a 1-1/2 inch layer of $Mg(ClO_4)_2$ followed by a 1/4 inch layer of silica gel, the latter also turning pink when damp. However, this chemical packing must be changed after running every ten samples. The dessicant tube should be installed whenever the samples contain less than 0.5 micrograms of total mercury. At higher mercury levels, use of the dessicant is optional. The tube should never be changed while running a batch of samples.

There are other methods of venting the mercury vapor from the system if use of an exhaust hood is not feasible. One alternative is illustrated in Figure 9. When a sample is being run, of course, clamps are placed at positions A and B. To eliminate the mercury from the lines, remove these clamps and place one at position C. The vapor is forced to pass through the scrubber, a plastic tube packed loosely with glass wool at each end and with activated charcoal in the middle. The charcoal does need to be replaced fairly often. Another method would be to bubble the vapor through a solution containing equal volumes of 0.1 M $KMnO_4$ and 10% H_2SO_4, or through a 0.25% I_2 in 3% KI solution. All of these methods have their own merits, but it generally takes 4-5 minutes to expel all the

Figure 8. Expanded Drawing of the Dessicant Tube[55]

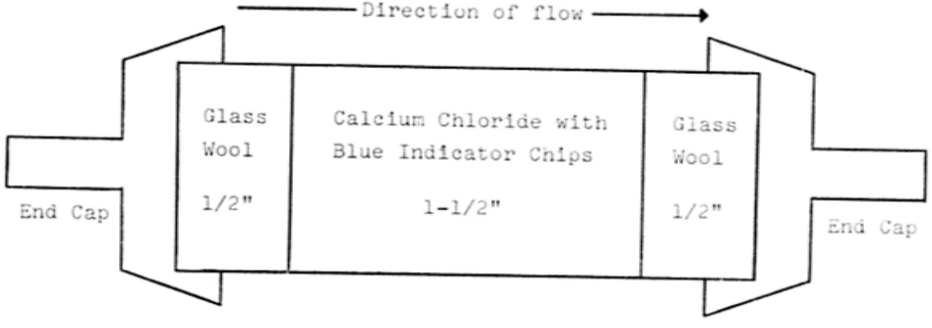
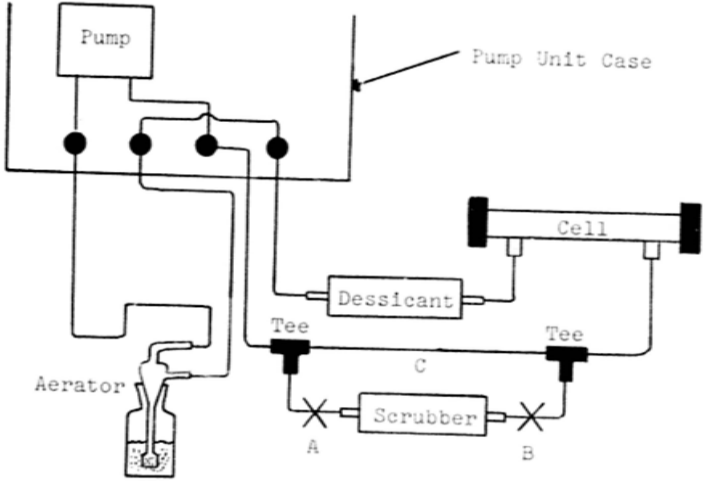

Direction of flow
Glass Wool 1/2"
Calcium Chloride with Blue Indicator Chips 1-1/2"
Glass Wool 1/2"
End Cap
End Cap

Figure 9. A Method for Venting Mercury in the Absence of an Exhaust Hood[55]


Pump
Pump Unit Case
Cell
Dessicant
Tee
Tee
C
Aerator
Scrubber
A
B

mercury from the lines, as compared to 1-2 minutes for an exhaust system.

The readings taken on the Perkin-Elmer 303 instrument were in percent absorption (%A). All of these were converted to absorbance (A) by use of the following equation:

$$A = -\log \frac{100-\%A}{100}$$

This is, of course, necessary since the mercury content is directly proportional to absorbance and not percent absorption.

C. Digestion of Fish Samples

The following procedure was used to digest the fish samples and to prepare a solution for mercury analysis. The amounts of reagents used are based on a 3g fish sample.

Place the entire goldfish sample in a 250 ml round-bottom flask equipped with a reflux condenser. Add 1.0 ml of 6 M HCl (checked for mercury content) and 15 ml concentrated HNO_3. Heat the samples to just below the boiling point and maintain that temperature for an hour. Allow the flask to cool and rinse the inside of the reflux condenser with deionized water, allowing the rinsings to enter the digestion flask. The solution should not be turbid. Filter off any undissolved bones and fat and dilute the solution to 50 ml with deionized water.

The above procedure proved to be satisfactory in obtaining fish solutions. Occasionally there were some undissolved bones and fat, but these have been shown not to retain any mercury.[48]

IV. RESULTS AND DISCUSSION

A. Choosing a Method for Flameless Analysis of Aqueous Samples

The first method attempted was one outlined by Perkin-Elmer in their instructions accompanying the mercury analysis system purchased.[55] A 100 ml sample thought to contain between 0 and 9 micrograms of mercury is transferred to a 300 ml BOD bottle and is treated with enough 5% $KMnO_4$ solution to keep the solution dark purple in color. Five ml of 30% HNO_3 are added and swirled. After 15 seconds an equal amount of 50% H_2SO_4 is added and swirled. Five ml of $NH_2OH \cdot HCl$ are added to reduce any excess permanganate, noted by a colorless solution within 15 seconds. Otherwise $NH_2OH \cdot HCl$ crystals are added to achieve this. Five ml of 10% $SnCl_2$ in 0.5 N H_2SO_4, a strong reducing solution, are then added and the aerator is immediately placed in the sample bottle. The sample absorbance is recorded.

A series of solutions containing known amounts of mercury as $HgCl_2$ were prepared and run using the above procedure. The calibration curve obtained is shown in Figure 10. A linear relationship is implied even up to 1.0 µg Hg. Since the mercury in these standards is already in the inorganic divalent state, this run tells us nothing of the method's ability to oxidize univalent or organic mercury. The results are vastly different when standard solutions containing mercury as PhHgCl were run, as shown in Figure 11. The curve is essentially

Figure 10. Calibration Curve for Inorganic Mercury ($HgCl_2$) Using the Permanganate Method

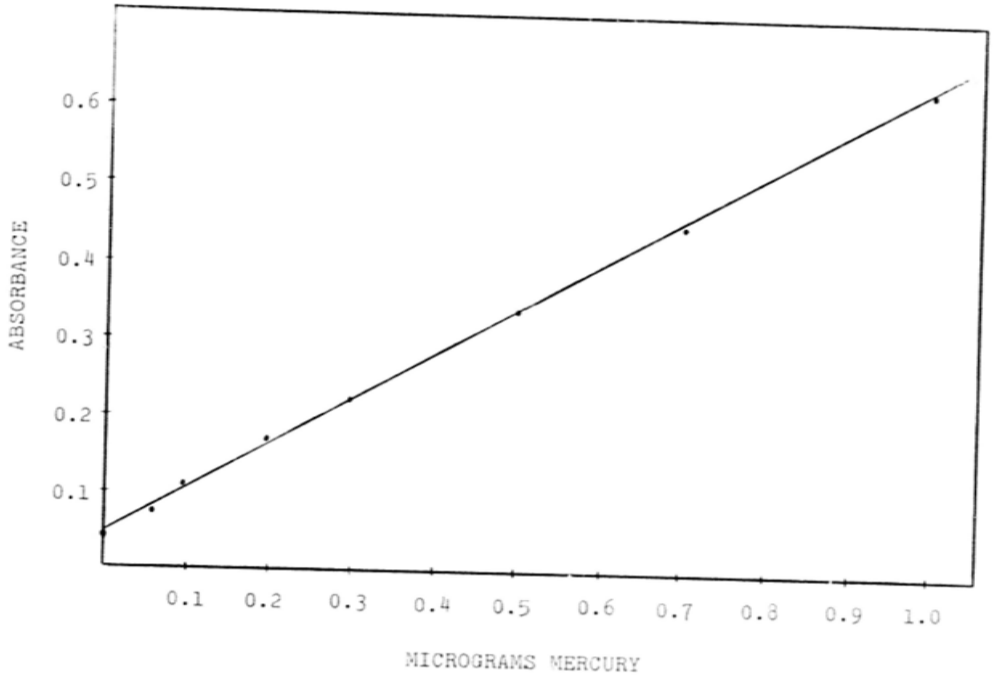
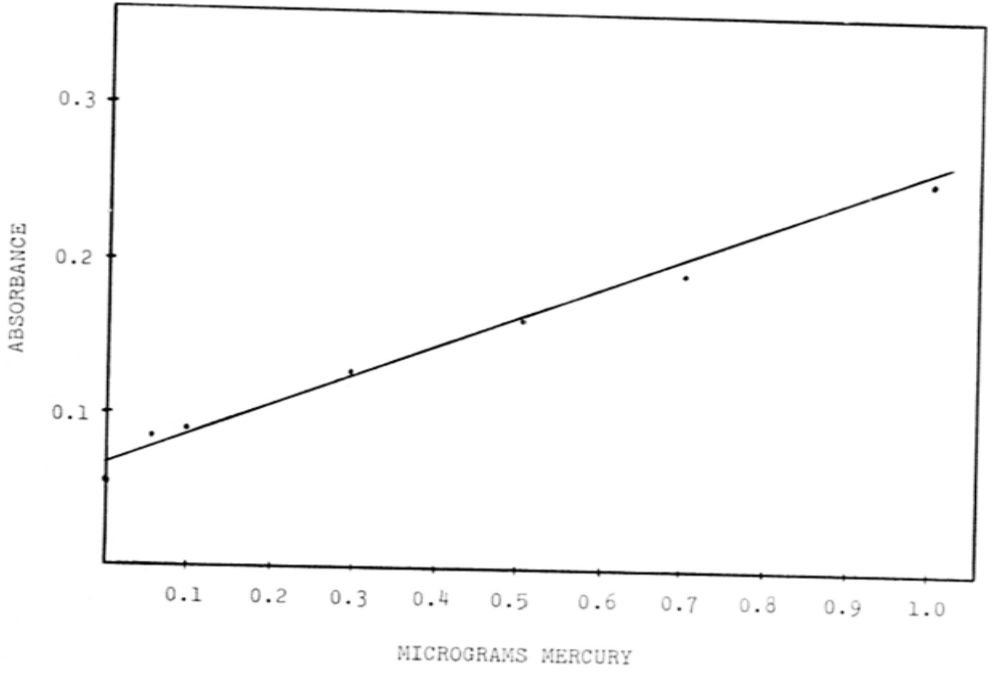

ABSORBANCE
0.6
0.5
0.4
0.3
0.2
0.1
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
MICROGRAMS MERCURY

Figure 11. Calibration Curve for Organic Mercury (PhHgCl) Using the Permanganate Method


0.3
0.2
0.1
ABSORBANCE
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
MICROGRAMS MERCURY

linear, but the absorbance values are less than half those obtained using inorganic divalent mercury. These curves were reproducible, indicating that stronger oxidizing conditions are needed to ensure that all mercury is in the unbound, divalent state.

There is a claim in the literature that $KMnO_4$ will only partially oxidize a number of organomercurials including phenylmercuric acetate and methylmercuric chloride.[56] The Environmental Protection Agency (EPA) method for analysis of mercury in water incorporates the use of $K_2S_2O_8$ along with $KMnO_4$ and a heat step to promote more complete oxidation. In light of this information, the following method was used.

Transfer a 100 ml sample containing between 0 and 0.5 µg Hg to a 300 ml BOD bottle and treat with 5 ml each of concentrated nitric and sulfuric acids. Add 20 ml 5% $KMnO_4$ and wait 15 minutes. Add more 5% $KMnO_4$ if the sample solution is not dark purple. Add 10 ml 5% $K_2S_2O_8$ and heat the sample for 1-1/2 hours in a water bath maintained at 85°C. Cool and add 8 ml 1.5% $NH_2OH \cdot HCl$ to reduce any excess permanganate. Add 5 ml 10% $SnCl_2$ in 0.5N H_2SO_4 and immediately attach the aerator to the sample bottle. Record the sample absorbance.

Using this method, a series of inorganic and organic mercury standards were run as before. The calibration curves are shown in Figures 12 and 13. It is apparent that this method is excellent for oxidizing organomercurials such as phenyl mercuric chloride. The absorbance values are about 90% of those obtained using $HgCl_2$. Comparison of Figures 10 and 12

Figure 12. Calibration Curve for Inorganic Mercury ($HgCl_2$) Using the Permanganate-Persulfate Method

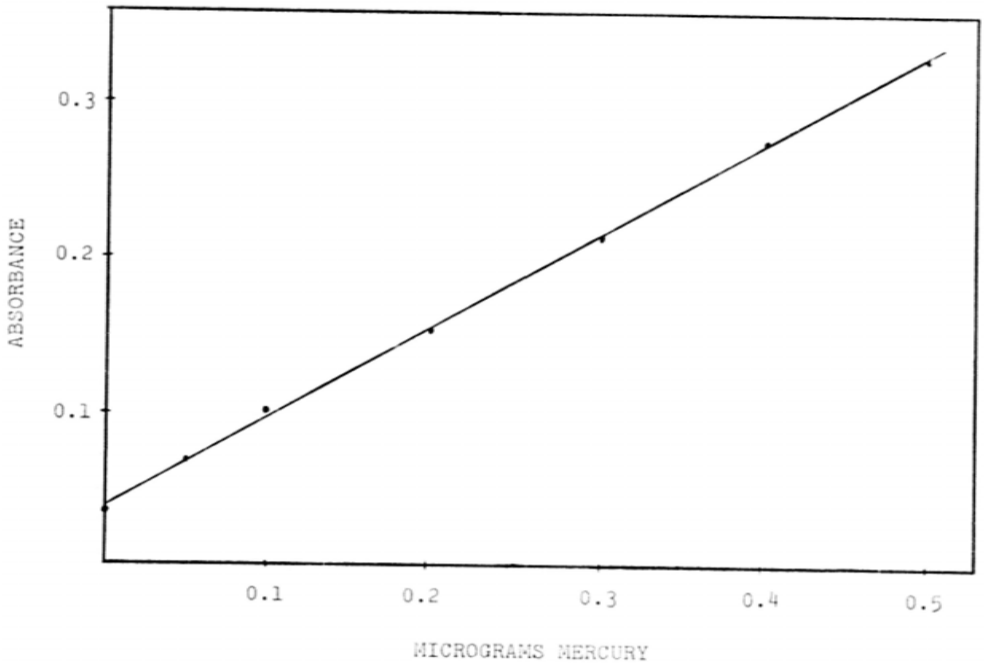
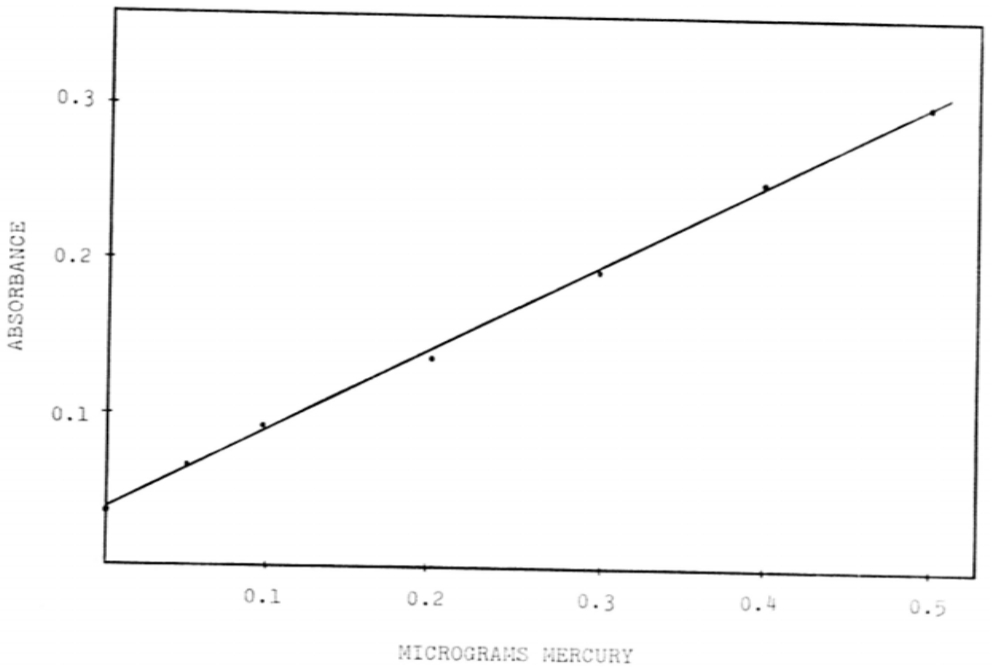

ABSORBANCE
0.3
0.2
0.1
0.1
0.2
0.3
0.4
0.5
MICROGRAMS MERCURY

Figure 13. Calibration Curve for Organic Mercury (PhHgCl) Using the Permanganate-Persulfate Method


0.3
0.2
0.1
ABSORBANCE
0.1
0.2
0.3
0.4
0.5
MICROGRAMS MERCURY

90% of those obtained using $HgCl_2$. Comparison of Figures 10 and 12 show that the absorbance values for $HgCl_2$ are nearly identical for the two methods, thus suggesting that mercury losses due to heating in the persulfate method are negligible. Based on these results, use of combined permanganate and persulfate as oxidizing agents is the method of choice.

When running a set of samples it is always best to run a reagent blank--that is, a solution of 100 ml of mercury-free water that has been treated in exactly the same manner as the samples. High blank absorbance values usually indicate that one or more of the reagents used is contaminated with mercury. The blank values obtained thus far have corresponded to an absorbance of about 0.040, which is somewhat higher than desirable. It was found that the major source of the problem was the $SnCl_2$ reagent. After only a day the reagent begins to turn from its original white color to a dull yellow, possibly due to oxidation of the stannous ion. If the solution is prepared fresh the average blank value drops to only 0.020. It is strange that the high blank value is seemingly unrelated to mercury contamination.

B. Results of Mercury-Fish Experiments

The purpose of our fish experiments was to test the effectiveness of the ylide triphenylphosphoniumcyclopentadienylide ($CpPPh_3$) in removing mercury from exposed goldfish. To accomplish this we placed equal numbers of goldfish, ranging in size from 5 to 7 cm, in aquaria containing only water. No rocks or vegetation were added so as to keep the number of experimental variables to a minimum. After allowing the system

to equilibrate a sublethal dose of $HgCl_2$ was added to each tank. After the fish absorbed most of the mercury a stoichiometric amount of $CpPPh_3$ was added to one of the tanks. It was our hope that the fish would consume the ylide, which would in turn complex some of the mercury contained within the fish and later be expelled as the mercury-ylide complex. The effectiveness of the ylide would be tested by taking groups of fish from each tank at convenient intervals and by observing any differences in mercury content of ylide-treated and untreated fish. Hopefully these differences would be very large. A total of three such experiments were performed.

Experiment 1

The first fish experiment was conducted as a trial run. Each tank held 54 gallons (205 ℓ), the large volume obviated the need for aeration. Only 15 goldfish were adued to each tank. One gram of fish food was added daily. Room temperature was maintained at 22°C. Enough $HgCl_2$ was added so that the initial mercury concentration was 0.31 ppm. A stoichiometric amount of ylide (0.1036 g) was added several hours after the mercury.

The results of this initial experiment are shown in Table 3. Since this run was for trial purposes only, just one fish was taken from each tank at the times shown. These crude results indicated that the fish in the ylide-treated tank had slightly lower mercury levels than their untreated counterparts. The mercury concentration in the water was also monitored to check the mercury uptake rate of the fish, which appeared to be quite rapid.

TABLE 3. Results of First Mercury-Fish Experiment

time of mercury exposure	mercury concentration, untreated tank (ppm)	mercury concentration, treated tank (ppm)
17 hours	FISH: 43.0 WATER: 0.100	FISH: 19.3 WATER: 0.050
65 hours	FISH: 51.1 WATER: 0.040	FISH: 27.4 WATER: 0.036
170 hours	FISH: 51.5 WATER: 0.020	FISH: 35.5 WATER: 0.016

Although the mercury levels in fish were comparable to those found by McKone, the copious production of mucus on the fish gills previously reported was not observed in any case. Some of the fish in both tanks developed brown spots, with those in the treated tank to a lesser degree.

It is significant that the fish showed no outward signs of toxicity as a result of consuming the ylide. Of the 30 fish started with, only 4 died and two of these were in the untreated tank. The ylide was totally consumed within 3 hours of the addition time.

Experiment 2

The following experimental conditions were used: tank colume, 58 gallons (220 ℓ); number of goldfish, 22 per tank; room temperature, 22°C; initial mercury concentration in water, 0.200 ppm; feeding rate, 2 g of food per day per tank. A stoichiometric amount of ylide (0.0722 g) was added five hours after the mercury.

The data obtained for this experiment is shown in Table 4. The mercury was again being rapidly consumed and its concentration seemed to approach a limiting vlue in both tanks. To prolong the length of the experiment only two fish were taken each time. This was not good sampling technique if we wished to arrive at an "average" value for each sampling. However, we were interested only in observing large mercury differences between fish in the two tanks. The fish in the untreated tank seemed to have widely varying mercury levels for the first two sampling times. These differences were much more than those

TABLE 4. Results of Second Mercury-Fish Experiment

time of mercury exposure	mercury concentration, untreated tank (ppm)	mercury concentration treated tank (ppm)
5 hours	WATER: 0.069	WATER: 0.111
22 hours	FISH: 16.3, 65.7 WATER: 0.036	FISH: 15.7, 16.5 WATER: 0.038
72 hours	FISH: 15.3, 42.6 WATER: 0.032	FISH: 11.1, 17.1 WATER: 0.025
one week	FISH: 13.8, 15.9 WATER: 0.023	FISH: 17.6, 19.6 WATER: 0.018

observed by McKone. A possible explanation is that there was a wide variation in fish size for this particular lot. That is, the mercury uptake rate is somewhat dependent upon the size of the fish. The mercury levels did seem to become more uniform after a week of exposure. There was still no significant difference in the mercury levels of treated and untreated fish.

The four remaining fish were then pooled; two were placed in each of two 7.7 gallon (29 ℓ) tanks. A large excess of $CpPPh_3$ (0.5 g) was added to one of the tanks. One-half gram of food was added to each tank daily. One month later the fish were sampled; those in the treated tank contained 1.5 and 2.8 ppm of mercury, while the untreated fish contained 2.5 and 2.7 ppm. Again there was no significant difference in mercury levels.

Based on the results of this experiment a repetition was definitely in order. Attempts were made to obtain a more uniform lot of fish.

Experiment 3

The following experimental conditions were used: tank volume, 58 gallons (220 ℓ); number of goldfish, 35 per tank and all of nearly uniform size; room temperature, 22°C; initial mercury concentration in water, 0.300 ppm; feeding rate, 2 g of food per day per tank. A stoichiometric amount of ylide (0.1075 g) was added 21 hours after the mercury.

The results of this last fish experiment are shown in Table 5. There was not a wide variation in mercury levels for

TABLE 5. Results of Third Mercury-Fish Experiment

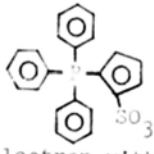
time of mercury exposure	mercury concentration, untreated tank (ppm)	mercury concentration, treated tank (ppm)
21 hours	FISH: 15.7, 19.9 WATER: 0.077	FISH: 21.9, 24.5 WATER: 0.103
71 hours	FISH: 9.5, 11.0 WATER: 0.011	FISH: 12.8, 13.3 WATER: 0.008
11 days	FISH: 8.2, 9.6 9.7, 10.6	FISH: 6.7, 7.8 8.9, 8.9
13 days	added 3.0 g more of $CpPPh_3$ to treated tank	
15 days	FISH: 10.3, 10.3 14.5, 15.0	FISH: 6.5, 7.3 9.1, 9.8
22 days	FISH: 8.1, 8.7 12.4, 12.7	FISH: 7.5, 9.3 14.9, 16.9
24 days	FISH: 10.0, 11.8	FISH: 9.3, 11.1

Seven of these fish were placed in each of two tanks, one containing only distilled water, the other distilled water treated with 2 g of sulfonated $CpPPh_3$. The results are seen below.

time of ylide exposure	mercury concentration, untreated tank (ppm)	mercury concentration, treated tank (ppm)
4 days	FISH: 5.1, 5.4	FISH: 3.6, 7.1
12 days	FISH: 3.3, 4.0	FISH: 4.1, 5.0

fish taken at a given sampling time, reflecting the importance of obtaining fish of uniform size. After seeing no appreciable difference in mercury levles for treated and untreated fish after 13 days, a huge amount of ylide (3.0 g) was added to the treated tank. After another 11 days there was still no change.

It was thought that the ineffectiveness of $CpPPh_3$ observed thus far might be a consequence of its insolubility in water. Perhaps it does not remain in the body of the fish long enough to coordinate mercury. A sulfonated version of $CpPPh_3$, sodium (2-triphenylphosphonium)cyclopentadienylide-sulfonate, was prepared and is shown below. It is quite water soluble and thus would remain in contact with the fish at all times. It might not, however, chelate mercury as effectively



as $CpPPh_3$ due to the electron-withdrawing sulfonium group.

The 14 remaining fish were pooled; seven were placed in each of two 7.7 gallon (29 ℓ) tanks. A large excess of sulfonated $CpPPh_3$ (2.0 g) was added to one of the tanks and dissolved rapidly. One-half gram of food was added daily. The fish showed no ill effects from exposure to the sulfonated ylide. The results are shown in Table 5 also.

C. Evaluation of $CpPPh_3$ as a Phase Transfer Catalyst

It was of interest to evaluate the ylide as a phase transfer catalyst (PTC). These phase transfer catalysts are used extensively to transfer anions across a phase boundary,[57]

and metal ion transport is well-known in biochemistry.[58] However, the only report[59] of cationic PTC to date was recently disputed.[60]

The reaction chosed for the study of this concept is shown below:

$$PhSH + HgCl_2 \xrightarrow{CpPPh_3} (Ph\text{-}S)_2Hg + 2HCl$$

The method involved dissolving PhSH and the ylide in an organic solvent, adding an aqueous $HgCl_2$ solution, shaking and then analyzing aqueous aliquots periodically for mercury content. It was considered that the ylide, by being dipolar, would preferentially migrate to the interface, combine with the mercury and move into the organic layer, whereupon PhSH would remove the mercury from the ylide, thus completing the cycle. PhSH was chosen because it is not very water-soluble and because thiols are excellent chelating agents for mercury.

Four samples were prepared for each analysis, each bottle differing in the contents of the organic phase. One bottle contained only PhSH, another PhSH plus ylide, a third ylide only and the fourth solvent only. Each experiment was repeated at least three times.

Analyses of the mercury contents in the aqueous layer indicate that there was no enhancement of mercury removal by the ylide in $CHCl_3$, CH_2Cl_2, CCl_4 or $PhNO_2$. The results in tetrahydrofuran/CCl_4 and in anisole/CCl_4 are quite different and are listed in Table 6.

The data in Table 6 indicates that PTC occurs in THF and in anisole. The most striking result is in THF where

TABLE 6. PTC Studies in THF and Anisole

Solvent	Sample	ppm Hg (aqueous layer)
75%THF, 25% CCl_4	PhSH	1560
	PhSH + ylide	560
	ylide	785
	solvent	655
75% $PhOCH_3$, 25% CH_2Cl_2	PhSH	1380
	PhSH + ylide	940
	ylide	1700
	solvent	1930

Note: All samples shaken for 30 seconds on an Eberbach water bath shaker.

it appears that PhSH and the ylide are individaully inhibitors of a process in which the mercury dissolves in the THF layer, but in combination, mercury transport is facilitated.

Despite considerable effort, no catalytic carrier has been found. When PhSH is added to the mercury-ylide complex, no precipitate of $(PhS)_2Hg$ forms. In fact, if the mercury-ylide complex and PhSH are combined in up to a 1:2 ratio and then $HgCl_2$ is added, no precipitate is formed. These observations are interpreted to signify tnat the PhSH coordinates with the mercury-ylide complex but that it is unable to bring about its decomposition to ylide and HgS.

The lack of an identifiable catalytic species brings us to the conclusion that the ylide interacts in some manner other than as a phase transfer catalyst. Perhaps its role is to change the interface surface tension.

IV. SUMMARY

Cold vapor flameless atomic absorption spectrophotometry is an excellent method for determination of mercury in trace quantities. The best method for analysis of aqueous samples involves the use of $KMnO_4$ and $K_2S_2O_8$ to promote the complete oxidation of both inorganic and organic mercury compounds to the unbound mercury(II) state. Acidic $SnCl_2$ effectively reduces mercury(II) to the vapor state.

The ylide triphenylphosphoniumcyclopentadienylide ($CpPPh_3$) quantitatively coordinates mercury in the presence of many other physiologically important metal ions. It is not effective, however, in removing mercury from exposed goldfish. This is perhaps due to its insolubility in water. A sulfonated version of $CpPPh_3$, although water soluble, also shows no promise in removing mercury from fish--though, initially, it may not chelate mercury as strongly as $CpPPh_3$.

A possible extension of this research would involve preparation of a sulfonated $CpPPh_3$ where the sulfonium group is not bonded to the cyclopentadienide ring. The sulfonium group would impart water solubility to the ylide without affecting its ability to strongly coordinate mercury. The fish experiments should then be repeated using this new ylide.

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